Platelet serotonin content and transpulmonary platelet serotonin gradient in patients with pulmonary hypertension

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Abstract: Background: The serotonin system has repeatedly been associated with the pathogenesis of pulmonary hypertension (PH). Objective: To comparatively analyze plasmatic and intrathrombocytic serotonin levels in arterial and mixed venous blood of patients with PH and unaffected controls to elucidate pulmonary serotonin metabolisms. Patients and Methods: Catheters were placed in the radial and pulmonary artery in patients with PH (n = 13) for diagnosis and in age-matched controls (n = 6) undergoing percutaneous closure of the patent foramen ovale. Arterial and mixed venous blood samples were immediately centrifuged to obtain plasma and platelets and thereafter frozen at -20 degrees C. After careful thawing, plasmatic and platelet serotonin levels were determined by ELISA. Results: PH was classified as arterial in 4 and chronic thromboembolic in 9 patients with a mean pulmonary artery pressure of 37 (interquartile range: 32-43) mm Hg. Platelet serotonin content was significantly lower in the PH patients than in the controls. The mean transpulmonary gradient (arterial-mixed venous) was negative in the PH group and positive in the controls. An inverse correlation was found between the arterial blood platelet serotonin content and pulmonary hemodynamics. Plasmatic serotonin levels did not differ between the PH and control groups. Conclusion: The lower platelet serotonin concentration in PH patients compared with unaffected controls is an unprecedented finding. The negative transpulmonary platelet serotonin gradient and the strong negative correlation of arterial blood platelet serotonin with pulmonary hemodynamics might indicate increased serotonin uptake in the lungs of PH patients.

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Platelet Serotonin Content and Transpulmonary Platelet Serotonin Gradient in Patients with Pulmonary Hypertension

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Key Words

Chronic thromboembolic pulmonary hypertension · 5-hydroxytryptamine · Platelets · Pulmonary hypertension · Serotonin uptake

Abstract

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Introduction

Pulmonary hypertension (PH) comprises a group of relatively rare disorders with a consistently progressive course and a dismal prognosis [1]. In the last decade, major advances in the pathogenetic understanding of pulmonary vascular diseases have led to better treatment options and prognosis [2]. These progresses are also reflect-

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ed in newer PH classifications and evolving therapies [2, 3]. Nevertheless, the major part of the etiopathogenesis of PH is still incompletely understood.

The serotonin system seems to be involved in the pathogenesis of PH. In the 1960s, an association between PH and the anorexigen aminorex was identified, leading to the withdrawal of aminorex from the market in 1972 [4]. In the 1980s, fenfluramine was the second anorexigen linked to PH [5]. These anorexigens (fenfluramine, D-fenfluramine and aminorex) have been shown to increase the levels of circulating serotonin (5-hydroxytryptamine, 5-HTT) by inhibiting its reuptake in neurons and platelets, inducing platelet release of serotonin, and by blocking its degradation through interaction with the monoamine oxidase enzymes [6–8]. Serotonin is a potent pulmonary vasoconstrictor but causes profound vasodilation in the systemic vasculature [7–9]. The different effects of serotonin on the two circulatory systems are similar to those observed under hypoxemia. Moreover, the effects of serotonin are intensified under hypoxemic conditions and the administration of catecholamines [8, 10]. Serotonin also plays an important role in pulmonary vascular remodeling and proliferation [11–14]. An increase in plasma serotonin associated with PH was first described in a patient with a rare hereditary thrombocytopathy, a defect in the serotonin-storing δ-granules [15]. Shortly thereafter, serotonin was found to be increased in the plasma of PH patients [16]. In in vivo studies, intravenous serotonin treatment of chronically hypoxic rats for 2 weeks increased pulmonary artery pressure, right-ventricular hypertrophy and pulmonary vascular remodeling [17]. The increased plasma serotonin was ascribed to platelet activation, which induces the release of the serotonin stored in granules into the extracellular space [18]. A pathogenetic role of platelet activation in PH patients was supported by the clinical efficacy of prostacyclins [19], which are potent inhibitors of platelet activation. However, elevated plasma serotonin levels did not correlate with platelet activation, and treatment with epoprostenol did not affect plasma serotonin levels in a later study [20].

Serotonin is an endogenous vasoactive indolamine found mainly in enterochromaffin tissue, brain and blood platelets. Approximately 90% of serotonin is located in the intestines, where it plays a key role in mechano- and chemotransduction. Most of the serotonin released from enterochromaffin cells reaches the portal circulation, where it is avidly taken up and stored in small electron-dense granules within platelets [21]. Platelet half-life of serotonin is in the range of 33–48 h; plasma serotonin is either catabolized by monoamine oxidase A in the liver, or taken up and actively transported via an integral membrane protein, the serotonin transporter (5-HTT), to the lung to be degraded inside endothelial and smooth muscle cells by monoamine oxidase B. These processes result in very low levels of free serotonin in plasma under normal physiological conditions [22].

As serotonin seems to play a crucial role in pulmonary vasoconstriction and proliferation and the lung is the major organ to metabolize serotonin produced in enterochromaffin cells of the gastrointestinal tract, we hypothesized that possible differences in the serotonin content in platelets and platelet-free plasma (PPF) in mixed venous and arterial blood between PH patients and healthy controls may add interesting information concerning the serotonin metabolism and its role in PH.

Patients and Methods

Consecutive patients scheduled for a diagnostic right-heart catheterization at the PH clinic of the University Hospital of Zurich, Switzerland, were prospectively included upon written informed consent. PH was defined as mean pulmonary artery pressure (mPAP) ≥25 mm Hg with a pulmonary capillary occlusion pressure ≤15 mm Hg. Patients were grouped according to the WHO classification into pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Diagnostic right-heart catheters (Swan Ganz; Edwards, Switzerland) were placed via a jugular vein and a radial arterial catheter. Cardiac output was assessed by thermodilution (Baxter/Edwards, USA). Hemodynamic data were assessed according to standard procedures and noted (mPAP, pulmonary capillary occlusion pressure, cardiac index, pulmonary vascular resistance, PVR, and arterial and mixed venous oxygen pressures and tensions). After a 10-min rest, radial arterial and mixed venous blood samples (5 ml of each) were atraumatically collected into citrate tubes. Similarly collected arterial and mixed venous blood samples from age-matched control patients with normal mPAP and PVR referred for heart catheterization due to other diseases (mainly patent foramen ovale closure due to the occurrence of transient ischemic attacks) served as controls. The blood was immediately transferred to the laboratory and centrifuged at 200 *g* at room temperature for 10 min to obtain platelet-rich plasma (PRP), and in 0.5 ml thereof platelets were counted and thereafter adjusted to ~350,000/μl; 300 μl PRP was added to 2.7 ml of 0.9 NaCl and centrifuged at 4,500 *g* at 4°C for 10 min. The supernatant was discarded and 600 μl sterile bidistilled water was added to the pellet. This amount was aliquoted in two tubes (150 ml each) and frozen at –20°C. The remainder of the plasma from the first centrifugation was again centrifuged at 4,500 *g* at 4°C for 10 min to obtain platelet-poor plasma (PPP) and frozen at –20°C.

The frozen aliquots of PRP and PPP simultaneously obtained from arterial and mixed venous blood were defrosted on ice and serotonin was determined by competitive ELISA after derivatization of serotonin to N-acetylserotonin (IBL; Germany). The transpulmonary serotonin contents in plasma and platelets were

Table 1. Patient characteristics

	PH	Controls
Patients, n (%)	13 (100)	6 (100)
Females/males, n (%)	10/3 (77/23)	2/4 (33/67)
Age, years	67 (60–73)	61 (53–67)
PH classification, n (%)		
PAH	4 (31)	
CTEPH	9 (69)	
mPAP, mm Hg	37 (32–43)	12 (11–13)
PVR, dyn·s·m^{-5}	640 (500–861)	107 (89–109)
Cardiac index, l/min/m^2	2 (1.8–2.4)	2.8 (2.7–2.8)
Mixed venous oxygen saturation	51 (50–56)	75 (75–76)
NYHA class I/II/III/IV, n (%)	0/2/8/3 (0/15/62/23)	1/0/0/0
6-min walking distance, m	450 (400–500)	not performed

Data are medians (interquartile ranges) and numbers (%).

Table 2. Serotonin (ng/ml) in plasma and platelets

	PH (n = 13)	Controls (n = 6)
PPF, mixed venous	5.4 (4.0–7.9)	6.3 (5.5–6.9)
PPF, arterial	6.3 (3.1–7.1)	6.6 (5.3–6.6)
Transpulmonary plasma gradient	–0.3 (–1.0–0.9)	–0.1 (–0.7–0.2)
Platelets, mixed venous	41 (36–74)*	99 (80–152)
Platelets, arterial	39 (27–52)**	160 (117–178)
Transpulmonary platelet gradient	–9.5 (–20–6.5)	30 (7–41)

Medians (quartile ranges). * $p = 0.013$, ** $p < 0.001$, vs. controls. There was no significant difference in the transpulmonary platelet gradient between the PH and control groups ($p = 0.32$).

calculated as follows: arterial minus respective mixed venous serotonin content.

Results are expressed as medians and interquartile ranges. The Mann-Whitney U test was used to compare means between groups. Pearson's correlation and scatter plots were used to show relationships between parameters. Differences in $p < 0.05$ were considered statistically significant. Statistical analysis was performed using SPSS (version 17.0).

The study was approved by the local ethical review board (University of Zurich, Switzerland).

Results

Thirteen patients with PH and 6 controls were included in the study. Patient characteristics are shown in table 1. Patients were mostly female, in New York Heart Association class III and were severely exercise limited (median 6-min walk distance 450 m, range 400–500). Four patients suffered from PAH (WHO class I; 2 idiopathic and 2 associated with connective tissue disease) and 9 patients had thromboembolic disease (WHO class IV). The two groups did not differ in clinical, hemodynamic and measured blood parameters, thus we analyzed them together. All 6 controls had normal pulmonary hemodynamics. Plasma serotonin levels in arterial and mixed venous blood were similar in PH patients and controls. Similarly, the transpulmonary plasma serotonin gradient (arterial serotonin minus mixed venous serotonin) did not differ between patients and controls (table 2). However, the platelet serotonin level was significantly lower in PH patients compared with controls, particularly in arterial blood. The transpulmonary platelet serotonin content was negative in PH patients, whereas it was positive in controls. We found a strong overall negative correlation of pulmonary hemodynamics with the arterial platelet serotonin content ($R = –0.850$, $p < 0.001$

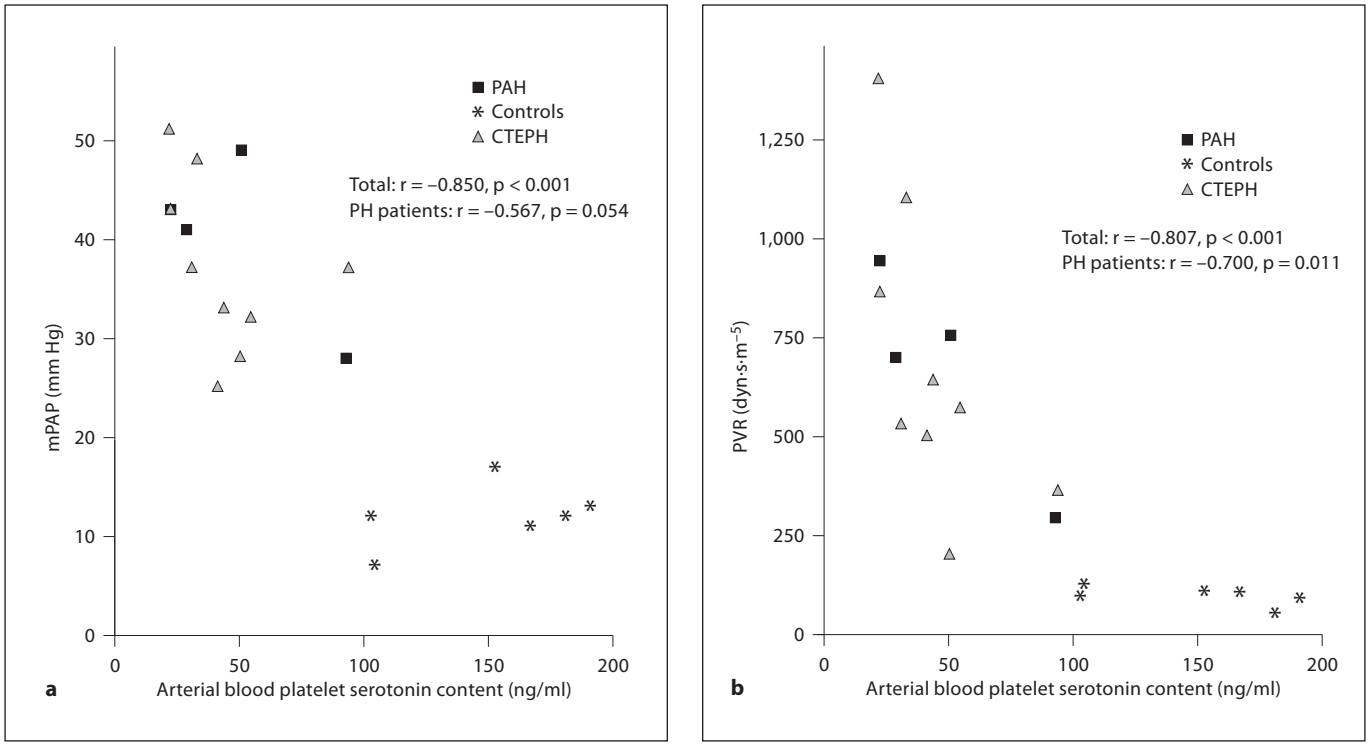

Fig. 1. Scatter plots showing a negative correlation of the arterial blood platelet serotonin content with mPAP (**a**) and PVR (**b**). Pearson's R and p values are shown in the figure for total study group (patients and controls) and PH patients alone.

for mPAP and R = –0.807, $p < 0.001$ for PVR, fig. 1) and the mixed venous platelet serotonin content (R = –0.566, p = 0.18 for mPAP and R = –0.636, p = 0.006 for PVR). For the arterial blood, this correlation persisted even after looking only at PH patients (R = –0.567, p = 0.054 for mPAP and R = –0.700, p = 0.011 for PVR, fig. 1).

Discussion

Our results firstly demonstrate a lower platelet serotonin content in PH patients compared with unaffected controls, especially in the arterial blood, resulting in a negative transpulmonary platelet serotonin content in PH patients, whereas it was positive in unaffected controls.

The serotonin system seems to play a role in PH, but the exact mechanisms remain to be elucidated [23]. Most probably, all three components of the serotonin system (synthesis, transporter and receptors) seem to be involved via a complex interaction and regulation system. Although elevated plasma serotonin levels have been found previously in PH patients compared with healthy controls [16], a direct link between plasma serotonin and PH could not be established [20]. In our study, plasma serotonin levels did not significantly differ between the PH patients and the controls. The major reason for these differences may be that the plasma half-life of serotonin is very short. Conversely, we found a decreased platelet serotonin content in PH patients compared with controls. To our knowledge, platelet serotonin levels have not been described in PH patients so far. Since the half-life of platelet serotonin is considerably longer than the half-life of plasma serotonin, it might represent a more robust measurement parameter. Although reference values of platelet serotonin levels in healthy controls have scarcely been published, gender differences have not been reported but levels seem to decrease with age [24]. As patients and controls were matched for age, we can exclude this confounder in our study. Interestingly, the difference in the platelet serotonin content between patients and controls was most pronounced in the arterial blood, resulting in a negative transpulmonary platelet gradient in PH, whereas in controls this gradient was positive. It could therefore be

hypothesized that in PH patients serotonin is consumed or degraded in the lung, either through enhanced monoamine oxidase activity or increased uptake by the stressed endothelium, e.g. by increased serotonin uptake by 5-HTT on pulmonary vascular endothelial and smooth muscle cells, with increased 5-HTT expression on these cells in PAH patients compared with controls [25–27]. However, we do not know why platelet serotonin values were considerably higher in arterial compared with mixed venous blood in controls. Thus, further studies unraveling the mechanisms of serotonin metabolism in the healthy and diseased human lung are warranted. Furthermore, a strong negative correlation was found between the arterial platelet serotonin content and pulmonary hemodynamics. This correlation even persisted, when we looked only at PH patients. This inverse correlation between pulmonary hemodynamics and the arterial platelet serotonin content might indicate that the strain on the pulmonary vessel wall due to PH might lead to an increased degradation of platelet serotonin. However, further research including in vivo models is needed to better elucidate the pathophysiological function of the serotonin system in PH.

Our study is limited by the small sample size and methodological issues. However, PH is a rare disease and immediate processing of patient blood obtained during right-heart catheterization is a logistical challenge. Moreover, as we processed the blood of patients and controls similarly, any methodological flaw should have been equally distributed among patients and controls. We included patients with PAH and CTEPH in our study, conditions with elevated pulmonary artery pressures due to different etiologies. It remains to be determined to date whether the involvement of the serotonin system differs depending on the etiology of PH and whether the etiology of PH might have affected the results. However, in our small study we did not find differences in blood serotonin levels between PAH and CTEPH. It is open to discussion whether patients with patent foramen ovale comprise an optimal control group for PH patients and it cannot be excluded that some of the differences found might be attributed to a different serotonin metabolism in the controls. However, it would have been ethically difficult to catheterize healthy people since all controls included lacked exercise limitation or dyspnea and had normal pulmonary hemodynamics.

In summary, our finding of decreased platelet serotonin in PH compared to unaffected controls is novel. Together with the clearly negative correlation of arterial blood platelet serotonin with pulmonary vascular pressure and resistance, these findings may point towards increased intrapulmonary serotonin uptake in PH.

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